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(54) Title: POLYHYDROXYALKANOATE COMPOSITIONS HAVING CONTROLLED DEGRADATION RATES

(57) Abstract

Biocompatible polyhydroxyalkanoate compositions, particularly based on biotechnologically produced homo- and copolymers of 4- hydroxybutyric acid, with controlled degradation rates have been developed. In one embodiment, the polyhydroxyalkanoates contain additives like, for example, pore forming agents to alter the degradation rates. In another embodiment, the polyhydroxyalkanoates are formed of mixtures of monomers or include pendant groups or modifications in their backbones to alter their degradation rates. In still another embodiment, the polyhydroxyalkanoates are chemically modified. Methods for manufacturing the devices which increase porosity or exposed surface area can be used to alter degradability. As demonstrated by the examples, these polyhydroxyalkanoate compositions have extremely favorable mechanical properties, as well as are biocompatible and degrade within desirable time frames under physioogical conditions. These polyhydroxyalkanoate materials provide a wider range of polyhydroxyalkanoate degradation rates than are currently available. Methods for processing these materials, particularly for therapeutic, prophylactic or diagnostic applications, or into devices which can be implanted or injected, are also described.

POLYHYDROXYALKANOATE COMPOSITIONS HAVING CONTROLLED DEGRADATION RATES

Field of the Invention

The present invention generally relates to polyhydroxyalkanoate
polymers and methods for altering their rates of degradation, particularly
methods which accelerate this process, and novel biodegradable
polyhydroxyalkanoates which are particularly suited for medical
applications.

Background of the Invention

In the medical area, a number of degradable polymers have been
developed that breakdown *in vivo* into their respective monomers within
weeks or a few months. Despite the availability of these synthetic
degradable polymers, there is still a need to develop degradable polymers
which can further extend the range of available options. In particular there is
a need to develop degradable polymers which offer a wider range of
mechanical properties.

Polyhydroxyalkanoates are natural, thermoplastic polyesters and can
be processed by traditional polymer techniques for use in an enormous
variety of applications, including consumer packaging, disposable diaper
linings and garbage bags, food and medical products. Initial efforts focused
on molding applications, in particular for consumer packaging items such as
bottles, cosmetic containers, pens, golf tees and the like. U.S. Patent Nos.
4,826,493 and 4,880,592 describe the manufacture of PHB and PHBV films
and their use as diaper backsheet. U.S. Patent No. 5,292,860 describes the
manufacture of the PHA copolymer poly(3-hydroxybutyrate-co-3-
hydroxyhexanoate) and the use of these polymers for making diaper
backsheet film and other disposable items. Diaper back sheet materials and
other materials for manufacturing biodegradable or compostable personal
hygiene articles from PHB copolymers other than PHBV are described in
PCT WO 95/20614, WO 95/20621, WO 95/23250 WO 95/20615, WO
95/33874, US 5,502,116, US 5,536,564, US 5,489,470 and WO 96/08535.

One of the most useful properties of PHAs which readily
distinguishes them from petrochemical derived polymers is their

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biodegradability. Produced naturally by soil bacteria, the PHAs are degraded upon subsequent exposure to these same bacteria in either soil, compost, or marine sediment. Biodegradation of PHAs is dependent upon a number of factors such as the microbial activity of the environment and the surface area

5   of the item. In addition, temperature, pH, molecular weight and crystallinity are important factors. Biodegradation starts when microorganisms begin growing on the surface of the plastic and secrete enzymes which break down the polymer into hydroxy acid monomeric units. The hydroxy acids are then taken up by the microorganisms and used as carbon sources for growth. In

10   aerobic environments the polymers are degraded to carbon dioxide and water, whereas in anaerobic environments the degradation products are carbon dioxide and methane (Williams, S.F. and Peoples, O.P., *CHEMTECH*, 26, 38-44 (1996)). While the mechanism for degradation of PHAs in the environment is widely considered to be via enzymatic attack

15   and can be relatively rapid, the mechanism of degradation *in vivo* is generally understood to involve simple hydrolytic attack on the polymers' ester linkages. It may or may not be protein mediated. Unlike polymers comprising 2-hydroxyacids like polyglycolic and polylactic acid, the polyhydroxyalkanoates are normally comprised of 3-hydroxyacids and in

20   certain cases even 4, 5, and 6-hydroxyacids. Ester linkages derived from these hydroxyacids are generally less susceptible to hydrolysis than ester linkages derived from 2-hydroxyacids.

Researchers have developed processes for the production of a great variety of PHAs and around 100 different monomers have been incorporated

25   into polymers under controlled fermentation conditions (Steinbüchel, A. and Valentin, H.E., *FEMS Microbiol., Lett.*, 128:219-228 (1995)). There are currently only two commercially available PHA compositions, poly-(R)-3-hydroxybutyrate (PHB) and poly-(R)-3-hydroxybutyrate-co-(R)-3-hydroxyvalerate (PHBV). Because of their great compositional diversity,

30   PHAs with a range of physical properties can be produced (Williams, S.F. and Peoples, O.P., *CHEMTECH*, 26:38-44 (1996)). The commercially available PHAs, PHB and PHBV, represent only a small component of the property sets available to the PHAs. For example, the extension to break of

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PHB and PHBV range from around 4 to 42%, whereas the same property for poly-4-hydroxybutyrate (P4HB) is about 1000% (Saito, Y. and Doi, Y. *Int. J. Biol. Macromol.* (1994) 16: 99-104). Similarly, the values of Young's modulus and tensile strength for PHB and PHBV are 3.5 to 0.5 GPa and 40

5   to 16 MPa, respectively (for increasing HV content to 25 mol %), compared to 149 MPa and 104 MPa, respectively for P4HB (Saito, Y. and Doi, Y. Int. J. Biol. Macromol. (1994) 16: 99-104).

In addition to finding commercial use as a biodegradable replacement for synthetic commodity resins, PHB and PHBV have been extensively

10   studied for use in biomedical applications. These studies range from potential uses in controlled release which have been reviewed by Koosha, F. *et al., Crit. Rev. Ther. Drug Carrier Syst.* 6:117-130 (1989) and Pouton C.W. and Akhtar, S. *Adv. Drug Delivery Rev.,* 18:133-162 (1996), to use in formulation of tablets, surgical sutures, wound dressings, lubricating

15   powders, blood vessels, tissue scaffolds, surgical implants to join tubular body parts, bone fracture fixation plates, and other orthopedic uses, as described in WO 98/51812 by Metabolix. Perhaps the most advanced medical development is the use of PHB and PHBV for preparing a porous, bioresorbable flexible sheet for tissue separation and stimulation of tissue

20   regeneration in injured soft tissue described in European Patent Application 754 467 A1 to Bowald, S. and Johansson-Ruden, G. filed June 26, 1988 and EP 0349505 A2. Recent reports have also described the use of PHBV to sustain cell growth (Rivard, C.H. *et al., J. Appl. Biomat.,* 6:65-68 (1995)).

Besides biocompatibility, it is often desired that an implanted medical

25   device should degrade after its primary function has been met. PHB and PHBV, the only PHAs tested as medical implants to date, have shown very long *in vivo* degradation periods, of greater than one year for PHB (Duvernoy, O., Malm, et al. *Thorac. Cardiovasc. Surgeon* (1995) 43: 271-74. Malm, et al., C. *J. Thorac. Cardiovasc. Surg.* (1992) 104: 600-607.). For

30   many applications, this very long degradation time is undesirable as the persistence of polymer at a wound healing site may lead to a chronic inflammatory response in the patient. Slowly degrading PHB patches used to regenerate arterial tissue have been found to elicit a long term (greater

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than two years) macrophage response (Malm, et al.,. *Eur. Surg. Res.* 1994,

26: 298-308). Macrophages were identified as being involved in the

degradation of the PHB implants and this long term macrophage response

appears to indicate the presence of persistent, slowly degrading particulate

5   material originating from the implant. Indeed, although a PHB patch used

for repair of the pericardium was not seen by ordinary light microscopy after

12 months implantation, small residual particulate material was observed by

polarized light microscopy (Malm, et al., C. *Scand. J. Thor. Cardiovasc.*

Surg. 1992, 26; 9-14). It is not clear if this particulate material remains

10   localized at the implant site, or may migrate throughout the body, causing

unforeseen complications. The biological fate, or medical impact of this

particulate material, cannot be predicted without long term study. In order to

minimize potential problems associated with slowly degrading PHAs, it is

advantageous to utilize resorbable materials with faster *in vivo* degradation

15   rates.

There has been only one report describing the biocompatibility or *in*

vivo degradation of any other PHA polymer in biomedical applications (WO

98/51812). U.S. Patent No. 5,334,698 to Witholt, B. and Lageveen, R.G.

mentions medical articles manufactured with an optically active polyester

20   isolated from *Pseudomonas oleovorans* cells, however, no examples or

discussion of fabrication or biocompatibility testing are cited, and no

methods are provided to obtain the polymer in a suitably pure form for *in*

vivo medical use. Since bacteria suitable for production of these polymers

may also produce an endotoxin as well as other inflammatory mediators, it is

25   important that the polymer be processed to remove these contaminants.

For many applications, the rate of PHA biodegradation is well suited

to the required product lifetime. However, in certain cases it would be

desirable to be able to exert more control over the rate at which the polymers

breakdown in the environment. Such control would extend the range of

30   applications for this class of polymers. For example, a PHA film may have

suitable mechanical properties to be used as a mulch film, yet not have the

most optimum rate of degradation for the application. The ability to be able

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to control the rate of degradation of the polymer in the environment would thus be a distinct advantage.

Thus while the polyhydroxyalkanoates offer a wide range of mechanical properties which are potentially useful in medical applications, their use particularly *in vivo* as resorbable polymers has been limited by their slow hydrolysis. It would thus be desirable to develop methods for controlling the rates of degradation of polyhydroxyalkanoates.

It is therefore an object of this invention to provide methods for controlling the rates of degradation of polyhydroxyalkanoates.

It is further object of this invention to provide new compositions comprising or derived from polyhydroxyalkanoates which degrade more readily in the environment and/or *in vivo*.

It is another object of this invention to provide methods for fabricating articles and devices from these compositions.

Summary of the Invention

Biocompatible polyhydroxyalkanoate compositions with controlled degradation rates have been developed. In one embodiment, the polyhydroxyalkanoates contain additives to alter the degradation rates. In another embodiment, the polyhydroxyalkanoates are formed of mixtures of monomers or include pendant groups or modifications in their backbones to alter their degradation rates. In still another embodiment, the polyhydroxyalkanoates are chemically modified. Methods for manufacturing the devices which increase porosity or exposed surface area can be used to alter degradability. For example, as demonstrated by the examples, porous polyhydroxyalkanoates can be made using methods that creates pores, voids, or interstitial spacing, such as an emulsion or spray drying technique, or which incorporate leachable or lyophilizable particles within the polymer. Examples describe poly(4HB) compositions including foams, coatings, meshes, and microparticles. As demonstrated by the examples, these polyhydroxyalkanoate compositions have extremely favorable mechanical properties, as well as are biocompatible and degrade within desirable time frames under physioogical conditions. These polyhydroxyalkanoate materials provide a wider range of

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polyhydroxyalkanoate degradation rates than are currently available.

Methods for processing these materials, particularly for therapeutic, prophylactic or diagnostic applications, or into devices which can be implanted or injected, are also described.

5   Brief Description of the Drawings

Figure 1 is a schematic of PHA biopolymers broadly divided into groups according to the length of their pendant groups and their respective biosynthetic pathways.

Figure 2a is a schematic of the pathways by which short pendant
10   group PHAs are derived. Figure 2b is a schematic of the pathways by which long pendant group PHAs are derived.

Figure 3 is a graph of P4HB degradation *in vivo* over time (weeks).

                            Detailed Description of the Invention

I.   PHA Compositions

15   *Polymer Compositions*

As used herein, "PHA materials" contain one or more units, for example between 10 and 100,000, and preferably between 100 and 30,000 units of the following formula I:

$$-OCR^1R^2(CR^3R^4)_nCO-;$$

20   wherein n is an integer, for example between 1 and 15, and in a preferred embodiment, between 1 and 4; and

wherein R^1, R^2, R^3, and R^4 independently can be hydrocarbon radicals including long chain hydrocarbon radicals; halo- and hydroxy-substituted radicals; hydroxy radicals; halogen radicals; nitrogen-substituted
25   radicals; oxygen-substituted radicals; and/or hydrogen atoms.

As used herein, the formula $-(CR^3R^4)_n-$ is defined as including the following formulas:

$-CR^3R^4-$ (where n=1);

$-CR^3R^4CR^{3'}R^{4'}-$ (where n=2); and

30        $-CR^3R^4CR^{3'}R^{4'}CR^{3''}R^{4''}-$ (where n=3);
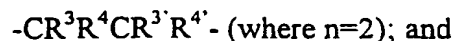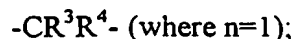
wherein R^3, R^4, $R^{3'}$, $R^{4'}$, $R^{3''}$, and $R^{4''}$ can be independently hydrocarbon radicals including long chain hydrocarbon radicals; halo- and hydroxy-substituted radicals; hydroxy radicals; halogen radicals; nitrogen-

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substituted radicals; oxygen-substituted radicals; and/or hydrogen atoms.
Thus, formula I includes units derived from 3-hydroxyacids (n=1), 4-
hydroxyacids (n=2), and 5-hydroxyacids (n=3).

These units may be the same in a homopolymer, or be more different

5   units, as for example in a copolymer or terpolymer. The polymers typically
have a molecular weight over 300, for example between 300 and 10^7, and in
a preferred embodiment 10,000 to 10,000,000 Daltons.

The PHA materials may contain or be modified to include other
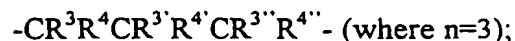molecules, such as bioactive and detectable compounds, surface active

10   agents, other degradable or non-degradable polymers, as well as materials
used to modify the mechanical properties of PHAs such as plasticizers,
fillers, nucleating agents, colorants, stabilizers, modifiers and binders.

Representative PHAs which can be modified or formulated as
described herein are described in: Steinbüchel, A. and Valentin, H.E., *FEMS*

15   *Microbiol., Lett.*, 128:219-228 (1995).

PHB and P4HB possess very different physical properties. A range
of PHA copolymers containing 4-hydroxybutyrate are either known or can
be prepared with a range of intermediate properties between those of PHB
and P4HB (Saito, Y. and Doi, Y. Int. J. Biol. Macromol. (1994) 16: 99-104),

20   however, biomedical applications, biocompatibility testing and *in vivo*
degradation of P4HB and its copolymers have not been reported. PHA
copolymers of 4HB and 3HB varying in composition from 0 to 100% 4HB
have been produced in *Alcaligenes eutrophus* (Nakamura, S., Doi, Y. and
Scandola, M. *Macromol.* (1992) 25:4237-4231) and from 64 to 100% 4HB in

25   *Comamonas acidovorans* (Saito, Y. and Doi, Y. *Int. J. Biol. Macromol.*
(1994) 16: 99-104), however, these polymers were of modest molecular mass
(1×10^5 to 5×10^5 g/mol, by GPC) compared to the molecular mass
produced in recombinant *E. coli* (greater than 5×10^5 g/mol, GPC).

The PHA biopolymers may be broadly divided into three groups

30   according to the length of their pendant groups and their respective
biosynthetic pathways (Figure 1). Those with short pendant groups, such as
polyhydroxybutyrate (PHB), a homopolymer of R-3-hydroxybutyric acid (R-
3HB) units, are highly crystalline thermoplastic materials, and have been

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known the longest (Lemoigne, M. and Roukhelman, N., *Annales des fermentations*, 5:527-536 (1925)). A second group of PHAs containing the short R-3HB units randomly polymerized with much longer pendant group hydroxy acid units were first reported in the early seventies (Wallen, L.L.

5   and Rohwedder, W.K., *Environ. Sci. Technol.*, 8:576-579 (1974)). A number of microorganisms which specifically produce copolymers of R-3HB with these longer pendant group hydroxy acid units are also known and belong to this second group (Steinbüchel, A. and Wiese, S., *Appl. Microbiol. Biotechnol.*, 37:691-697 (1992)). In the early eighties, a research group in

10   The Netherlands identified a third group of PHAs, which contained predominantly longer pendant group hydroxy acids (De Smet, M.J. *et al.*, *J. Bacteriol.*, 154:870-878 (1983)).

The PHA polymers may constitute up to 90% of the dry cell weight of bacteria, and are found as discrete granules inside the bacterial cells.

15   These PHA granules accumulate in response to nutrient limitation and serve as carbon and energy reserve materials. Distinct pathways are used by microorganisms to produce each group of these polymers. One of these pathways leading to the short pendant group polyhydroxyalkanoates (SPGPHAs) involves three enzymes, namely thiolase, reductase and PHB

20   synthase (sometimes called polymerase). Using this pathway, the homopolymer PHB is synthesized by condensation of two molecules of acetyl-Coenzyme A to give acetoacetyl-Coenzyme A, followed by reduction of this intermediate to R-3-hydroxybutyryl-Coenzyme A, and subsequent polymerization (Figure 2a). The last enzyme in this pathway, the synthase,

25   has a substrate specificity that can accommodate C3-C5 monomeric units including R-4-hydroxy acid and R-5-hydroxy acid units. This biosynthetic pathway is found, for example, in the bacteria *Zoogloea ramigera* and *Alcaligenes eutrophus*. The biosynthetic pathway which is used to make the third group of PHAs, the long pendant group polyhydroxyalkanoates

30   (LPGPHAs) is still partly unknown, however, it is currently thought that the monomeric hydroxyacyl units leading to the LPGPHAs are derived by the b-oxidation of fatty acids and the fatty acid pathway (Figure 2b). The R-3-hydroxyacyl-Coenzyme substrates resulting from these routes are then

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polymerized by PHA synthases (sometimes called polymerases) that have substrate specificities favoring the larger monomeric units in the C6-C14 range. Long pendant group PHAs are produced, for example, by *Pseudomonads*.

5   Presumably, the second group of PHAs containing both short R-3HB units and longer pendant group monomers utilize both the pathways shown in Figures 2a and 2b to provide the hydroxy acid monomers. The latter are then polymerized by PHA synthases able to accept these units.

          In all, about 100 different types of hydroxy acids have been
10   incorporated into PHAs by fermentation methods (Steinbüchel, A. and Valentin, H.E., *FEMS Microbiol., Lett.*, 128:219-228 (1995)). Notably, these include PHAs containing functionalized pendant groups such as esters, double bonds, alkoxy, aromatic, halogens and hydroxy groups.

          A preferred polyhydroxyalkanoate for medical applications is P4HB.
15   P4HB is biocompatible, resorbable, processable, strong and ductile. Maintenance of breaking strength is another very important parameter for suturing and stapling materials, especially resorbable ones. As resorbable materials are degraded *in vivo*, their physical and mechanical properties change as the result of this degradation. For instance, a resorbable suture
20   will loose most of its breaking strength, and as such its ability to fix tissue, more rapidly than the time for its complete resorption. PGA sutures, for example, will loose most of their strength within three weeks *in vivo* (Vet Surg 21;192:355-61), but not be completely resorbed before six weeks. This loss of mechanical strength is the result of molecular mass decrease of the
25   polymer. It is important to note that a number of parameters will affect resorption rates and suture breaking strength *in vivo*, such as type of tissue, mechanical stresses, presence of infection, etc.

          The examples demonstrate that the degradation rate of P4HB *in vivo* is fast relative to other PHAs, however, its resorption rate is slower than
30   many of the materials used as resorbable sutures. Additionally, as shown in Table 7, P4HB implants maintain their molecular mass during the process of resorption. This maintenance of molecular mass is expected to be a benefit for the maintenance of mechanical properties, and as such breaking strength

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of PHAs used as wound closing materials. Because of their excellent
mechanical properties, maintenance of high molecular mass, processability,
biocompatibility and resorbability, P4HB and P4HB-co-HA should be useful
as resorbable wound closure materials such as suturing and stapling

5   materials, particularly as modified herein to increase their degradation rates.

Sources of PHAs

PHA materials which can be modified to alter their degradation rates
can be derived from either a biological source, an enzymatic source, or a
chemical source. The biological source can be a microorganism or higher

10   organism such as a plant, and can be derived by genetic engineering.

During the mid-1980's, several research groups were actively
identifying and isolating the genes and gene products responsible for PHA
synthesis. These efforts lead to the development of transgenic systems for
production of PHAs in both microorganism and plants, as well as enzymatic

15   methods for PHA synthesis. Such routes could increase further the available
PHA types. These advances have been reviewed in Williams, S.F. and
Peoples, O.P., *CHEMTECH*, 26, 38-44 (1996), and Williams S.F. and
Peoples, O.P., *Chem. Br.* 33, 29-32 (1997).

Methods which can be used for producing PHA polymers suitable for

20   subsequent modification to alter their rates of degradation are described in:
U.S. Patent No. 4,910,145 to Holmes, P.A. and Lim, G.B.; Byrom, D.,
"Miscellaneous Biomaterials," in D. Byrom, Ed., "Biomaterials" MacMillan
Publishers, London, 1991, pp. 333-359; Hocking, P.J. and Marchessault,
R.H. "Biopolyesters", G.J.L. Griffin, Ed., "Chemistry and Technology of

25   Biodegradable Polymers," Chapman and Hall, London, 1994, pp.48-96;
Holmes, P.A., "Biologically Produced (R)-3-hydroxyalkanoate Polymers and
Copolymers," in D.C. Bassett Ed., "Developments in Crystalline Polymers,"
Elsevier, London, Vol. 2, 1988, pp. 1-65; Lafferty *et al.*, "Microbial
Production of Poly-b-hydroxybutyric acid," H.J. Rehm and G. Reed, Eds.,

30   "Biotechnology", Verlagsgesellschaft, Weinheim, Vol. 66, 1988, pp. 135-
176; Müller and Seebach, *Angew. Chem. Int. Ed. Engl.* 32:477-502 (1993);
Steinbüchel, A. "Polyhydroxyalkanoic Acids," in D. Byrom Ed.,
"Biomaterials", MacMillan Publishers, London, 1991, pp. 123-213; and,

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Williams and Peoples, *CHEMTECH*, 26:38-44, (1996); Steinbüchel and

Wiese, *Appl. Microbiol. Biotechnol.*, 37:691-697 (1992); U.S. Patent Nos.

5,245,023; 5,250,430; 5,480,794; 5,512,669; 5,534,432; Agostini, D.E. *et al.*;

Polym. Sci., Part A-1, 9:2775-2787 (1971); Gross, R.A. *et al.*,

5   *Macromolecules*, 21:2657-2668 (1988); Dubois, P.I. *et al.*, *Macromolecules*,

26:4407-4412 (1993); Le Borgne, A. and Spassky, N., *Polymer*, 30:2312-

2319 (1989); Tanahashi, N. and Doi, Y., *Macromolecules*, 24:5732-5733

(1991); Hori, Y.M. *et al.*, *Macromolecules*, 26:4388-4390 (1993);

Kemnitzer, J.E. *et al.*, *Macromolecules*, 26:1221-1229 (1993); Hori, Y.M. *et*

10   *al.*, *Macromolecules*, 26:5533-5534 (1993); Hocking, P.J. and Marchessault,

R.H., *Polym. Bull.*, 30:163-170 (1993); Xie, W. *et al.*, *Macromolecules*,

30:6997-6998 (1997), and, U.S. Pat. No. 5,563,239 to Hubbs, J.C. and

Harrison, M.N. The PHAs derived from these methods may be in any form,

including a latex or solid form.

15   Identification, cloning and expression of the genes involved in the

biosynthesis of PHAs from several microorganisms within recombinant

organisms allow for the production of PHAs within organisms that are not

native PHA producers. Such recombinant organisms provide researchers

with a greater degree of control of the PHA production process because they

20   are free of background enzyme activities for the biosynthesis of unwanted

PHA precursors or degradation of the PHA. Additionally, the proper

selection of a recombinant organism may facilitate purification of, or allow

for increased biocompatibility of, the produced PHA.

            The minimal requirements for the synthesis of PHA in a recombinant

25   organism are a source of hydroxyalkanoyl-CoA and an appropriate PHA

synthase (Gerngross, T.U. and Martin, D.P. *Proc. Natl. Acad. Sci.* (1995) 92:

6279-6283.). Recombinant PHA producers, thus require a biosynthetic

pathway for a hydroxyalkanoyl-CoA monomer and a suitable PHA synthase.

Production of a homopolymer requires that the organism produce only one

30   suitable substrate for the PHA synthase, as production of multiple substrates

results in the formation of a PHA copolymer. Recombinant organisms

containing a transgene encoding a PHA synthase are sufficient for

production of P4HB.

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In the absence of PHA degradation pathways, the molecular mass of the PHA accumulated in recombinant organisms can be very high. PHB produced in recombinant *E. coli* has been reported to have molecular mass of 4×10^6 g/mol (Sim, S.J., Snell, K.D., Hogan, S.A., Stubbe, J., Rha, C. and

5 Sinskey, A. Nature Biotech. (1997) 15: 63-67). The molecular mass is important for controlling the physical properties of a given PHA, because the increased molecular mass of PHAs produced in recombinant organisms can lead to improved material properties, such as increased tensile strength and ultimate elongation (Kusaka, S., Iwata, T. and Doi, Y. *J.M.S. Pure Appl.*

10 *Chem.* (1998) A35:319-335).

The biosynthesis of P3HB-co-4HB containing a low level of 4HB (1.5%) has been described in recombinant *E. coli* (Valentin, H. E., Dennis, D. *J. Biotech.* 1997, 58; 33-38). It is noteworthy that the molecular mass of these PHAs were very high (greater than 1×10^6 g/mol). Additionally, the

15 biosynthesis of the P3HB-co-4HB and the homopolymer P4HB in recombinant *E. coli* have been described (Hein, et al.. *FEMS Microbiol. Lett.* 1997, 153; 411-418).

In addition to using biological routes for PHA synthesis, PHA polymers may also be derived by chemical synthesis. One widely used

20 approach involves the ring-opening polymerization of ß-lactone monomers using various catalysts or initiators such as aluminoxanes, distannoxanes, or alkoxy-zinc and alkoxy-aluminum compounds (see Agostini, D.E. *et al.,* Polym. Sci., Part A-1, 9:2775-2787 (1971); Gross, R.A. *et al.,* Macromolecules, 21:2657-2668 (1988); Dubois, P.I. *et al., Macromolecules,*

25 26:4407-4412 (1993); Le Borgne, A. and Spassky, N., *Polymer,* 30:2312-2319 (1989); Tanahashi, N. and Doi, Y., *Macromolecules,* 24:5732-5733 (1991); Hori, Y.M. *et al., Macromolecules,* 26:4388-4390 (1993); Kemnitzer, J.E. *et al., Macromolecules,* 26:1221-1229 (1993); Hori, Y.M. *et al., Macromolecules,* 26:5533-5534 (1993); Hocking, P.J. and Marchessault,

30 R.H., *Polym. Bull.,* 30:163-170 (1993). A second approach involves condensation polymerization of esters and is described in U.S. Patent No. 5,563,239 to Hubbs, J.C. and Harrison, M.N., and references therein. Researchers have also developed chemo-enzymatic methods to prepare

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PHAs. Xie *et al.*, *Macromolecules*, <u>30</u>:6997-6998 (1997), for example, have reported a ring opening polymerization of beta-butyrolactone by thermophilic lipases to yield PHB.

Biological production of P4HB or P4HB-co-HA has certain

5   advantages over traditional chemical synthetic methods. The chemical synthesis of high molecular mass P4HB (greater than 1×10^5 g/mol) is difficult due to the tendency of the free acid to lactonize to form the relatively unstrained and kinetically favored five-membered ring. Thus, polycondensation of 4-hydroxybutyric acid is difficult to achieve, while the

10   material that results from high pressure ring-opening polymerization reactions of γ-butyrolactone is of very low molecular mass (Korte, F. and Gelt, W. *Polymer Lett.* 1966, 4, 685) and would have poor mechanical properties. An alternate synthetic strategy for P4HB, the free radical ring-opening polymerization of 2-methylene dioxolane, results in a copolymer

15   containing ring opened and unopened units (Bailey,et al. *J. Polym. Sci. Polym. Chem.* (1982) 20: 3021-30. Bailey, W. *J. Polym. Preprints* (1984) 25:210-11.). 4HB has been successfully co-polymerized with 3HB via ring-opening polymerization (Hori, Y., Yamaguchi, A. and Hagiwara, T. *Polymer* 1996, 36, 4703-4705.), however, the molecular weight of the copolymers

20   was modest (less than 1×10^5 g/mol), especially for compositions with more than 80% 4HB (less than 2×10^4 g/mol). Additionally, many of the catalysts used for the chemical synthesis of polyesters contain toxic metals. These toxic contaminants can be avoided using a biological process to produce PHAs.

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II.   **PHA Formulations Having Altered Degradation Rates**

The degradation rates of the polymers can be manipulated through addition of various components to the polymeric composition, as well as selection of the chemical composition, molecular weight, processing

30   conditions, and form of the final polymeric product. The chemical composition can be altered through selection of monomers which are incorporated into the polymer, by alteration of the linkages, chemical backbone or pendant groups, and/or by manipulation of the molecular

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weight. Increasing porosity, inclusion of hydrophilic substances, and/or increasing surface area exposed to water, will all increase the rate of degradation. Hydrophobic coatings or incorporation into or blended with of hydrophobic substances with the polymers will decrease the rate of

5   degradation.

Additives Altering Degradation Rates

The hydrolysis of polyhydroxyalkanoates is accelerated at acidic or basic pH's and thus the inclusion of acidic or basic additives or excipients can be used to modulate the rate of degradation of PHAs. The excipients can

10   be added as particulates, can be mixed with any other additive or agent incorporated or to be incorporated, or can be dissolved within the polymer.

Additives which enhance the rate of degradation include inorganic acids such as ammonium sulfate and ammonium chloride, organic acids such as citric acid, benzoic acids, peptides, ascorbic acid, inorganic bases such as

15   sodium carbonate, potassium carbonate, calcium carbonate, zinc carbonate, and zinc hydroxide, and organic bases such as protamine sulfate, spermine, choline, ethanolamine, diethanolamine, and triethanolamine and surfactants such as Tween™ and Pluronic™. Such additives are preferably used at concentrations between 0.1 and 30% by weight.

20   The rate of degradation may also be enhanced by additives which form pores or otherwise increase the surface area in the polymer or increase the amorphous content of the polymer. Pore forming agents are generally added as particulates and include water soluble compounds such as inorganic salts and sugars which are removed by leaching. Suitable particles include

25   salt crystals, proteins such as gelatin and agarose, starches, polysaccharides such as alginate and other polymers. The diameters of the particles may suitably be between nanometers to 500 microns. They may also be lyophilizable. Pore forming agents can be included in an amount of between 0.01% and 90% weight to volume, preferably at a level between one and

30   thirty percent (w/w, polymer), to increase pore formation. For example, in spray drying or solvent evaporation, a pore forming agent such as a volatile salt, for example, ammonium bicarbonate, ammonium acetate, ammonium chloride or ammonium benzoate or other lyophilizable salt, is first dissolved

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in water. The solution containing the pore forming agent is then emulsified with the polymer solution to create droplets of the pore forming agent in the polymer. This emulsion is then spray dried or taken through a solvent evaporation/extraction process. After the polymer is precipitated, the

5   hardened microparticles are frozen and lyophilized to remove the pore forming agents. Plasticizers, such as the citrate esters, and other polymers like atactic polyhydroxyalkanoates, may be added to increase the polymer's amorphous character.

Hydrophobic coatings or materials which can be incorporated to
10   increase the degradation rates include hydrophobic compounds such as phospholipids, cholesterol, and other polymers, as well as surfactants. These materials and methods for forming coatings or incorporation into the materials are described in WO 96/18420 by Bracco Research SA, WO 92/18164 by Delta Biotechnology, Ltd., WO 95/03356 by Massachusetts
15   Institute of Technology, PCT/US97/03007 by Acusphere, U.S. Patent No. 5,271,961 to Mathiowitz, et al., U.S. Patent No. 5,711,933 to Bichon, et al., and U.S. Patent No. 5,705,187 to Unger. Specific examples disclose fatty acids and phospholipids as emulsifiers to stabilize the oil phase in the aqueous phase during emuslion/encapsulation process, with the result that
20   the microspheres arecoated with an outer layer of the surfactant. The use of additives such as fats, waxes, and high molecular weight hydrocarbon are also disclosed to hydrophobize the polymer walls and to slow water penetration.

Modification of PHA Pendant Groups

25   An alternative method to alter the rate of degradation of PHA polymers involves modification of the polyhydroxyalkanoate pendant groups. The pendant groups may be modified in whole or in part. Pendant groups can, for example, be converted to acidic and basic groups, such as carboxylic acids and amines. These types of groups can enhance degradation

30   by altering local pH values. Alternatively, the pendant groups can be converted to reactive groups, such as alcohols and amines, which can cleave the polymer backbone either by an intramolecular or intermolecular reaction. In addition to these conversions, the pendant groups may also be converted

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to hydrophilic groups to increase uptake of hydrolytic agents such as water, or they may be converted to groups which would increase the amorphous nature of the polymers. The procedures required to carry out functional group conversion of the pendant groups are well known to those skilled in
5   the art. One suitable method that can be used for preparing a PHA of the invention (incorporating a unit that alters the degradation rate of the polymer) is taught in WO 98/39453 by Hein, Sohling, Gottschalk, and Steinbuchel. Suitable pendant groups in PHA polymers which will alter the rate of degradation can also be derived directly by fermentation.

10   *Chemical Modification of PHAs*

        The rate of hydrolysis of a polyhydroxyalkanoate depends upon a number of factors. One key factor is the chemical nature or reactivity of the ester linkages between the monomers. The rate of degradation of the PHA backbone can thus be altered by incorporating into the polymer backbone
15   chemical linkages which are more susceptible to hydrolysis, or enzymatic attack. Examples of monomers which can be incorporated into polyhydroxyalkanoate backbones to alter the rate of degradation of the polymer are 2-hydroxy acids, such as glycolic acid and lactic acid, and other hydroxyacids which modulate the reactivity of the ester linkage, such as 2-
20   hydroxyethoxy acetic acid. Besides incorporating other hydroxyacids which yield esters which are more susceptible to hydrolytic or enzymatic attack, other types of functionality may be incorporated into the polymer backbone. For example, one or more of the ester linkages can be replaced by groups such as amide, anhydride, carbonate, or carbamate. Examples of monomers
25   which can be incorporated into the polyhydroxyalkanoate backbone are aminoacids and aminoalcohols. Moreover, multifunctional monomers can be incorporated into the polyhydroxyalkanoate backbones, for example, triols or tetraols. These types of monomer units can also be used to increase or maintain molecular weight of the polymer by interchain crosslinking, or
30   modify crystallinity of the polymers.

        A variety of methods may be used to incorporate susceptible chemical linkages into the polyhydroxyalkanoate backbones. For example, co-feeds may be added during fermentation of PHAs which result in the

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incorporation of desired monomers. Suitable co-feeds include
hydroxyalkoxy acetic acids. These types of monomers may also be
incorporated during chemical synthesis from hydroxyacid monomers using
catalysts, and via coenzyme A derivatives using enzymatic catalysts such as

5   the PHA synthases.

Susceptible chemical linkages may also be incorporated into
polyhydroxyalkanoate backbones after their initial synthesis. Methods to
accomplish this include chemical transformations such as insertion reactions,
irradiation, esterification, transesterification (see for example, Otera, J. *et al.*,

10   *Tetrahedron Lett.*, 27:2383-2386 (1986), Otera J. *et al.*, *J. Org. Chem.*, 56:
5307-5311 (1991), Otera, J. *et al.*, *J. Org. Chem.*, 54:4013-4014 (1989) and
Otera, J. *et al.*, *J. Chem. Soc., Chem. Commun.*, 1742-1743 (1991)), ester
metathesis reactions (see for example, Stanton, M.G. and Gagné, M.R., *J.
Am. Chem. Soc.*, 119:5075-5076 (1997) and references therein), and reactive

15   blending. In the latter case, chemical reactions can be carried out in the melt
with a catalyst present. For example, esters or polyesters can be melted with
polyhydroxyalkanoates in the presence of suitable catalysts in order to
chemically modify the polyhydroxyalkanoate.

Processing of PHAs Including Susceptible Linkages

20   The polyhydroxyalkanoates may be further manipulated using a wide
range of polymer processing techniques. Preferred methods for processing
these materials include: solvent casting, melt processing, fiber
processing/spinning/ weaving, extrusion, injection and compression molding,
and lamination.

25   **III.   Methods for Manufacturing Medical Devices**

The polymer compositions are useful for preparing a variety of
biodegradable medical devices. The biodegradable polymers preferably
exhibit a relatively slow biodegradation, for example, having a *in vivo* half-
life of between three and six months or less. The polymers preferably have a

30   relatively low melting point/glass transition temperature, for example, less
than 136°C, and/or are soluble in a non-toxic, non-halogenated solvent, for
ease of processing.

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When the depyrogenated PHAs are implanted in the body, these materials show very little, if any, acute inflammatory reaction or any adverse tissue reaction. There is no significant inflammatory response or scar tissue formation. Recruitment of inflammatory cells is minimal. Histological

5   examination of the explanted devices demonstrates that the materials are essentially inert. Accordingly, devices constructed of PHAs can be implanted with minimal adverse affect on the surrounding tissue. Release of the hydroxy acid degradation products from the implanted materials typically is slow and well tolerated by the body. Thus, PHAs are expected to

10   maintain their material properties for a matter of months and will eventually degrade to non-toxic materials.

Devices prepared from the PHAs can be used for a wide range of different medical applications. Examples of such applications include controlled release, drug delivery, tissue engineering scaffolds, cell

15   encapsulation; targeted delivery, biocompatible coatings; biocompatible implants; guided tissue regeneration, wound dressings, orthopedic devices, prosthetics and bone cements (including adhesives and/or structural fillers), and diagnostics.

The PHAs can encapsulate, be mixed with, or be ionically or

20   covalently coupled to any of a variety of therapeutic, prophylactic or diagnostic agents. A wide variety of biologically active materials can be encapsulated or incorporated, either for delivery to a site by the polyhydroxyalkanoate, or to impart properties to the polymer, such as bioadhesion, cell attachment, enhancement of cell growth, inhibition of

25   bacterial growth, and prevention of clot formation.

Examples of suitable therapeutic and prophylactic agents include synthetic inorganic and organic compounds, proteins and peptides, polysaccharides and other sugars, lipids, and DNA and RNA nucleic acid sequences having therapeutic, prophylactic or diagnostic activities. Nucleic

30   acid sequences include genes, antisense molecules which bind to complementary DNA to inhibit transcription, and ribozymes. Compounds with a wide range of molecular weight can be encapsulated, for example, between 100 and 500,000 grams or more per mole. Examples of suitable

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materials include proteins such as antibodies, receptor ligands, and enzymes,
peptides such as adhesion peptides, saccharides and polysaccharides,
synthetic organic or inorganic drugs, and nucleic acids. Examples of
materials which can be encapsulated include enzymes, blood clotting factors,

5   inhibitors or clot dissolving agents such as streptokinase and tissue
plasminogen activator; antigens for immunization; hormones and growth
factors; polysaccharides such as heparin; oligonucleotides such as antisense
oligonucleotides and ribozymes and retroviral vectors for use in gene
therapy. The polymer can also be used to encapsulate cells and tissues.

10   Representative diagnostic agents are agents detectable by x-ray,
fluorescence, magnetic resonance imaging, radioactivity, ultrasound,
computer tomagraphy (CT) and positron emission tomagraphy (PET).
Ultrasound diagnostic agents are typically a gas such as air, oxygen or
perfluorocarbons.

15   In the case of controlled release, a wide range of different bioactive
compounds can be incorporated into a controlled release device. These
include hydrophobic, hydrophilic, and high molecular weight
macromolecules such as proteins. The bioactive compound can be
incorporated into the PHAs in a percent loading of between 0.1% and 70%

20   by weight, more preferably between 5% and 50% by weight. The PHAs may
be in almost any physical form, such as a powder, film, molded item,
particles, spheres, latexes, and crystalline or amorphous materials. They can
be combined with additional non-PHA materials, for example, other
polymers. They are suitable for use in applications requiring slowly

25   degrading, biocompatible, moldable materials, for example, medical devices.
Examples of medical devices which can be prepared from the polymers
include rods, bone screws, pins, surgical sutures, stents, tissue engineering
devices, drug delivery devices, wound dressings, and patches such as hernial
patches and pericardial patches.

30   Degradable implants fabricated with the PHAs may be used in a wide
range of orthopedic and vascular applications, tissue engineering, guided
tissue regeneration, and applications currently served by other thermoplastic
elastomers (McMillin, *Rubber Chem. Technol.*, 67:417-46 (1994)). The

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implants may include other factors to stimulate repair and healing. Preferred devices are tubes suitable for passage of bodily fluids. These devices may be modified with cell attachment factors, growth factors, peptides, and antibodies and their fragments.

5   Preferred methods of fabricating medical devices include solvent casting, melt processing, extrusion, injection and compression molding, and spray drying. Particles are preferably prepared directly from a fermentation based process, or by a solvent evaporation technique, double emulsion technique, or by microfluidization, using methods available in the art.

10   (Koosha, F. Ph.D. Dissertation, 1989, Univ. Nottingham, UK., *Diss. Abstr. Int. B* 51:1206 (1990); Bruhn, B.W. and Müeller, B.W. *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.* 18:668-69 (1991); Conti, B. *et al., J. Microencapsulation,* 9:153-166 (1992); Ogawa, Y. *et al., Chem. Pharm. Bull.,* 36:1095-103 (1988); Mathiowitz, E. and Langer, R. "Polyanhydride

15   microspheres as drug delivery systems," M. Donbrow Ed., in "*Microcapsules Nanopart. Med. Pharm."* CRC, Boca Raton, Florida, 1992, Ch. 5, pp. 99-123.)

The PHAs can be fabricated into devices suitable for wound healing. For example, non-woven fibrous materials for this purpose may be prepared

20   from the polymers by first producing polymer fibers, by pressing the polymers through a perforated outlet, using procedures known to those skilled in the art. The fibers can then be fabricated into a porous membrane (cloth) by spreading them on a solid support and subjecting them to compression molding. The thickness of the device is preferably less than

25   500 μm. The wound healing device may also be prepared by perforating a film or membrane using a laser to achieve porosity, or using a leaching technique to prepare a porous material. The pore sizes should ideally be small enough to lock out cells and other tissue matter. The wound healing devices may be positioned *in vivo* to separate tissues and stimulate tissue

30   regeneration.

The PHAs may be used to encapsulate cells. Using procedures known to those skilled in the art, cells first may be pre-coated. Maysinger, *Reviews in the Neurosciences,* 6:15-33 (1995). Using a particle

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encapsulation procedure such as the double emulsion technique, the cells may then be encapsulated by PHAs. Ogawa, *et al.*, *Chem. Pharm. Bull.*, 36:1095-103 (1988). Encapsulated cells may then be implanted *in vivo*.

5   The PHAs may be fabricated into tissue engineering scaffolds using a wide range of polymer processing techniques. Preferred methods of fabricating PHA tissue engineering scaffolds include solvent casting, melt processing, fiber processing/spinning/weaving, extrusion, injection and compression molding, lamination, and solvent leaching/solvent casting. Such methods are known to those skilled in the art.

10   One preferred method of fabricating a PHA tissue engineering scaffold involves using an extruder, such as a Brabender extruder. For example, this technique can be used to prepare extruded tubes suitable for implantation in a range of lengths and sizes.

Another preferred method involves preparing a nonwoven PHA

15   scaffold from fibers. Fibers may be produced from the melt or solution, and processed into nonwovens using methods known to those skilled in the art. The properties of the nonwoven may be tailored by varying, for example, the PHA material, the fiber dimensions, fiber density, material thickness, fiber orientation, and method of fiber processing. The porous membranes may, if

20   desired, be further processed. For example, these membranes may be formed into hollow tubes.

Another preferred method involves melt or solvent processing a suitable PHA into an appropriate mold and perforating the material using a laser or other means to achieve the desired porosity. Also preferred are

25   methods that include rolling a compression molded PHA sheet into a loop and heat sealing. The PHA sheet optionally may be rolled with another material, such as a second biodegradable polymer. For example, the latter material could be a nonwoven of polyglycolic acid, polylactic acid, or a copolymer of glycolic and lactic acids. Such a procedure should provide a

30   laminated tube suitable for use in the engineering of new vessels, ducts and tubes. The PHAs may also be used to coat other tissue engineering scaffolds. Such materials could be derived from other degradable polymers. Coating

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may be performed, for example, with a solvent based solution, or by melt techniques, or using a PHA latex.

The tissue engineering devices described herein may be seeded with cells prior to implantation or after implantation. The cells may be harvested from a healthy section of the donor's tissue, expanded *in vitro* using cell culture techniques, and then seeded into a scaffold (or matrix) either prior to or after implantation. Alternatively, the cells may be obtained from other donor's tissue or from existing cell lines.

The PHAs may be used to coat other devices and materials. Such coatings may improve their properties for medical application, for example, improving their biocompability, mechanical properties, and tailoring their degradation and controlled release profiles. The PHAs may be coated onto other devices using the fabrication procedures described above. The thickness of the coating can be adjusted to the needs of the specific application by changing the coating weight or concentration applied, and/or by overcoating.

The PHAs may be fabricated into stents using a wide range of polymer processing techniques. Preferred methods of fabricating PHA stents include solvent casting, melt processing, fiber processing/spinning, extrusion, injection molding, and compression molding. Such methods are known to those skilled in the art.

Prior to implantation, a bioresorbable polymeric article must be sterilized to prevent disease and infection of the recipient. Sterilization is performed prior to seeding a polymeric device with cells. Heat sterilization of PHA containing articles is often impractical since the heat treatment could deform the article, especially if the PHA has a melting temperature below that required for the heat sterilization treatment. This problem can be overcome using cold ethylene oxide gas as a sterilizing agent. Exposure of a PHA containing article to vapors of ethylene oxide prior to implantation sterilizes the article making it suitable for implantation. During sterilization with cold ethylene oxide gas, the PHA containing article maintains its shape. This type of treatment is ideally suited for sterilization of molded, or pre-

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formed articles where the shape of the article plays in important role in its proper functioning.

The devices described herein can be administered systemically or locally, or even used *in vitro*, particularly for cell culture. The preferred

5   methods of systemically administering the devices are by injection, inhalation, oral administration and implantation. Other suitable methods for administering the devices include administering the devices topically, as a lotion, ointment, patch, or dressing.

The compositions and methods described herein will be further

10   understood with reference to the following non-limiting examples.

Example 1:   Production of P4HB in recombinant *Escherichia coli*

E. coli strain MBX1177, a derivative of strain DH5α selected for the ability to grow with 4-hydroxybutyric acid (4HB) as the sole carbon source, was transformed with pFS30, a plasmid containing the genes encoding PHA

15   synthase from *Ralstonia eutropha*, 4-hydroxybutyryl-CoA transferase from *Clostridium kluyveri*, and β-lactamase, which confers resistance to ampicillin. The synthase and transferase are under the control of the trc promoter, which is inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) in pFS30. These cells were first grown in 100 ml LB (Luria Broth, Difco,

20   Detroit, Mich.; 25 g/L) plus 100 μg/ml ampicillin overnight in a 250-ml Erlenmeyer flask at 37 °C with shaking at 200 rpm. This entire culture was used as an inoculum for the fermentation carried out in a 7L vessel. The first stage of the fermentation consisted of growing biomass in 5 L of LB-ampicillin at 37 °C with stirring at 800 rpm and aeration at 1 volumetric

25   volume of air/min (vvm). After 17 hours, the volume was adjusted to 6 L by adding one liter of medium, such that the total volume contained, per liter: 2.5 g LB powder, 5 g 4HB as sodium salt, 2 g glucose, 50 mmol potassium phosphate (pH 7), 7 g phosphoric acid, 100 μg ampicillin, and 0.1 mmol IPTG. At this time, the temperature was adjusted to 33 °C, and the agitation

30   rate was reduced to 400 rpm. Periodic additions of glucose and sodium 4HB were made when the pH was significantly below or above 7, respectively, because the addition of glucose caused the pH to decrease slowly and the addition of 4HB caused the pH to increase slowly. The pH was not

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automatically controlled. The fermentation proceeded this way for an additional 70 h, at which time a total of 34 g/L glucose and 15 g/L 4HB had been fed. The cells were allowed to settle at 4 °C for 2 days, after which time the liquid phase was pumped away, and the cell slurry was fluidized in a

5 Microfluidics Corporation (Newton, Mass.) M110-EH Microfluidizer at 18,000 psi. The resulting material was lyophilized and extracted into tetrahydrofuran (THF, 3% wt/vol P4HB) with heating (60°C) and mechanical stirring. The resulting THF extract was pressure filtered through glass micro-fiber (2.3 μm) and Teflon (2 μm) depth filters. The polymer was

10 precipitated into an equal volume of water and lyophilized. The polymer was redissolved in THF (3% wt/vol P4HB) with heating (60°C) and the solution was filtered through glass micro-fiber (2.3 μm) and Teflon (2 μm) depth filters and precipitated into water/THF (1:1). The precipitate was washed with water/THF (1:1) and lyophilize to yield a white colored foam

15 (20 g). This material was identified as poly-4-hydroxybutyrate and shown to be non-cytotoxic by an agar diffusion assay (ISO 10993, Toxicon Corp., Bedford, MA). Elemental analysis (C 55.63%, H 7.41%, O 37.28%, N 41 ppm). GC analysis shows very low lipids in the purified polymer. NMR analysis shows expected peaks and no lipids.

20 **Example 2:   Production of poly(4HB-co-2HB) in recombinant**
Escherichia coli.

     E. coli strains MBX1177/pFS30 and MBX184 (CGSC6966)/pFS30 were precultured in 300 mL LB-ampicillin in a one-liter Erlenmeyer flask at 30 °C overnight with shaking at 200 rpm. Two 100-mL aliquots of each

25 preculture were centrifuged (2000 x g, 10 minutes), and the cells obtained from each of these aliquots were resuspended in 100 mL of a medium containing, per liter: 6.25 g LB powder; 2 g glucose; 50 mmol potassium phosphate (pH 7); 100 μg ampicillin; and 100 μmol IPTG. The medium also contained 2-hydroxybutyric acid (2HB) and 4HB; in one flask the

30 concentrations were 8 g/L 2HB and 2 g/L 4HB, and in the other the concentrations of the two acids were each 5 g/L. Both acids were added to the flasks as the sodium salt; the masses given for the acids do not include the mass of sodium. These four flasks (two flasks for each strain) were

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incubated at 30 °C for an additional 48 hours with shaking at 200 rpm. The cells were removed from the medium by centrifugation (2000 x *g*, 10 minutes), washed once with water, centrifuged again, and lyophilized. Gas chromatographic analysis was carried out on the lyophilized cell mass to

5   analyze for polymer content and composition, see Table 2. The cellular contents and compositions of the PHAs produced are given in Table 2. When the ratio of 2HB to 4HB was 4:1, the 2HB content of the polymer was higher than 19 percent for both strains by GC analysis, while at a 1:1 ratio of 2HB to 4HB, the 2HB content of the polymer was around 1 percent. The

10   4HB was more readily incorporated into the polymer than was the 2HB; therefore, when 4HB was present at 2 g/L the overall polymer content of the cells is less than when it was present at 5 g/L. The polymers produced by MBX184/pFS30 were extracted from the cells and analyzed. The lyophilized cell mass was incubated in 5 mL of chloroform at 37 °C for 2

15   hours. The cell debris was removed by centrifugation (2000 x *g*, 5 minutes), and the resulting polymer solution was added dropwise to 50 mL of ethanol to precipitate it. The precipitated polymer was centrifuged from the ethanol as above. In the case of the 4:1 2HB:4HB ratio the polymer was difficult to centrifuge from the ethanol; it formed a haze when added to the ethanol but

20   not nearly all of it could be collected by centrifugation, probably because the molecular weight of this polymer was rather low. The polymer isolated from the 1:1 2HB:4HB flask was easily precipitated from the ethanol, and it was recovered nearly completely. GC analysis of these extracted samples (Table 2) showed that the 2HB content was slightly lower than when the analysis

25   was done on whole cells. It is possible that 2HB residues in the polymer chain are hydrolyzed during the extraction, thus lowering the apparent 2HB content in the extracted samples. The fact that the molecular weight of the extracted polymer was apparently lower when the 2HB content was higher is consistent with this explanation.

30   A second experiment was performed with MBX184/pFS30. These cells were precultured in 400 mL LB-ampicillin in a one-liter Erlenmeyer flask at 30 °C overnight with shaking at 200 rpm. An addition of 20 ml of medium was made to each flask such that the total volume contained, per

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liter: 2.5 g additional LB powder; 2 g 4HB as sodium salt; 2 g glucose; 50 mmol potassium phosphate (pH 7); 100 µg ampicillin; 50 µmol IPTG; and 2, 4, 6, or 8 g 2HB as sodium salt. The flasks were incubated for an additional 48 hours at 30 °C and 200 rpm. The cells were removed from the medium

5   by centrifugation (2000 x g, 10 minutes), washed once with water, centrifuged again, and lyophilized. The dried cell mass was subjected to GC analysis as above. Table 3 gives the cell content and composition of the polymers thus obtained. At low 2HB:4HB ratios, little or no 2HB was incorporated into the polymer; however, when this ratio was 3:1 or 4:1, 2HB

10   incorporation into the polymer was very significant. The overall polymer content of all the cells was rather low, probably because the acids are not present at concentrations high enough to permit the uptake and/or incorporation to proceed at a high rate.

Example 3:   Production of poly(4HB-co-3HB) in recombinant *E. coli.*

15   Strain MBX1177/pFS30 was precultured in 100 ml LB-ampicillin in four separate 250-ml Erlenmeyer flasks at 30 °C overnight with shaking at 200 rpm. An addition of 20 ml of medium was made to each flask such that the total volume contained, per liter: 2.5 g additional LB powder; 4 g 4HB as sodium salt; 4 g glucose; 50 mmol potassium phosphate (pH 7); 100 µg

20   ampicillin; 50 µmol IPTG; and 0.25, 0.5, 0.75, or 1 g 3-hydroxybutyrate (3HB) as sodium salt. The flasks were incubated for an additional 48 hours at 30 °C and 200 rpm. The cells were removed from the medium by centrifugation (2000 x g, 10 minutes), washed once with water, centrifuged again, and lyophilized. Gas chromatographic analysis was carried out on the

25   lyophilized cell mass to analyze for polymer content and composition. The standard used to test for 3-hydroxybutyrate units in the polymer was poly(3-hydroxybutyrate). The cellular contents and compositions of the PHAs produced are given in Table 4. As the ratio of 4HB/3HB in the medium decreased, the 3HB content of the polymer increased in a monotonic fashion,

30   while the overall polymer content of the cells was similar in all trials. This means that the composition of the medium can be used predictably to control the copolymer composition without significantly affecting the overall polymer yield. The polymer was extracted from the remainder of the

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lyophilized cell mass. For all samples, lyophilized cell mass was mixed with about three times its own volume of 1,2-dichloroethane and incubated with mild shaking in a closed tube at 37°C for 6 hours. The particulate matter was separated from the polymer solution by centrifugation (2000 x g, 10

5   minutes). The resulting solution was added dropwise to about 10 times its own volume of ethanol, and the precipitated polymer was allowed to settle out of solution. The supernatant was poured off, and the remaining wet polymer was allowed to stand until it appeared to be dry, then it was lyophilized to complete dryness. Thermal properties of these P4HB-co-3HB

10   compositions are shown in Table 5.

Example 4:   *In vitro* and *in vivo* **Degradation of P4HB.**

The degradation of P4HB was studied *in vitro* and *in vivo*. Three different configurations of varying porosity (0%, 50% and 80% porosity) were examined. Small disks (5 mm diameter) were punched from

15   compression molded P4HB films of uniform thickness. Porous samples of P4HB were produced using the salt leaching technique describedbelow. The degradation behavior *in vitro* was studied by incubating the disks in a sterile, phosphate buffer (8 mM sodium phosphate, 2 mM potassium phosphate, 140 mM NaCl, 10 mM KCl, pH 7.4, containing NaN_3 as preservative) at 37°C.

20   The degradation behavior *in vivo* was studied after implantation in subcutaneous pockets in rats.

Preparation of porous P4HB Classified sodium chloride crystals (80-180 μm) were mixed with molten P4HB. The polymer salt ratio can be adjusted to produce the desired porosity, while particle size may be adjusted to

25   produce pores of varying size. The polymer salt mixture was pressed into a thin film. After allowing the material to solidify, the film was removed from the mylar backing. The film was exhaustively extracted with water to remove the salt, leaving a porous film of P4HB.

Accelerated Degradation of P4HB The degradation of P4HB was studied *in*

30   *vivo*. Three different configurations of varying porosity (0%, 50% and 80% porosity) were examined. Small disks (5 mm diam.) were punched from compression molded P4HB films of uniform thickness. Porous samples of P4HB were produced using a salt leaching technique. The degradation

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behavior *in vivo* was studied after implantation in subcutaneous pockets in
rats. Samples were removed at various times. The molecular mass was
measured by GPC and mass loss was measured by quantification of the
remaining 4HB by CG analysis. The results are shown in Figure 3. As

5   shown in Figure 3, the sample mass loss varied with porosity. Film, 50%
and 80% porous samples showed a 5%, 20% and 75% mass loss,
respectively, over the six week period, while the average molecular mass loss
of these samples also decreased significantly (20 to 50%). These data
demonstrate that the degradation rate of PHAs can be modified and

10   controlled by altering porosity and increasing surface area.

Results

The P4HB implants showed a very minimal inflammatory response,
much less so than for a PGA non-woven mesh. This is a very good
indication of the biocompatibility of these materials. Samples were removed

15   at various times and evaluated histologically both as to the implants and
surrounding tissue. The molecular mass was measured by GPC and mass
loss was measured by quantification of the remaining 4HB by GC analysis.
The results are shown in Tables 6 and 7. As shown in Table 6, P4HB does
not degrade significantly over a ten week period *in vitro*. All of the samples

20   maintained their starting weight and there was about a 20 to 40% decrease in
average molecular mass. The samples incubated *in vivo* showed much more
pronounced degradation. The mass loss varied with porosity. Film, 50%
and 80% porous samples showed a 20%, 50% and 100% mass loss,
respectively, over the ten week period, while the average molecular mass

25   loss of these samples also decreased significantly (20 to 50%).

Light microscopic and environmental scanning electron microscopy
(ESEM) examination of the samples show almost no discernible change for
the *in vitro* samples over the ten week incubation period. On the other hand,
the *in vivo* implants show distinct signs of degradation. The surface of these

30   materials becomes progressively degraded during the ten week implantation
period. After one week, the film samples show some signs of cracking and
crazing, which progresses to surface erosion and pitting over the following
nine weeks.

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The *in vitro* degradation data suggest that P4HB is fairly stable to simple hydrolysis, unlike other polyesters used in bioresorbable applications, such as PGA, PLA and their copolymers. However, the degradation of the implants indicates that P4HB can be degraded *in vivo*, suggesting a

5   biologically mediated mode of degradation. The data shows increasing degradation with increasing porosity, which indicates that surface area of the polymer implant plays a role in its degradation *in vivo*. This suggests that the degradation of P4HB polymers *in vivo* occurs at the surface of the implant, unlike PGA or PLA materials which degrade throughout the implant

10   by hydrolysis, with associated molecular mass decrease and loss of mechanical properties. These data suggest that the degradation rate of P4HB can be modified and controlled by altering its surface area. Also, it is expected that this type of surface degradation will result in a relatively slow rate of molecular mass loss allowing for the maintenance of polymer material

15   properties longer than existing absorbable, medical polyesters. The P4HB implants were very well tolerated and showed only a very minimal foreign body reaction. These findings show that these materials have significant advantages over existing biomedical polyesters.

Example 5:   Compression molding.

20   P4HB was pressed into a thin film using Carver hydraulic press. The platens were heated to 115°C. P4HB was pressed between two sheets of mylar using metal spacers. Spacer thickness and pressure of the press can be adjusted to control film thickness. The film was removed from the press and allowed to cool at room temperature. After solidifying (within a matter of

25   seconds), the film was easily peeled from the mylar backing material. Mechanical data for this material are shown in Table 1. The rapid solidification of P4HB demonstrates it rapid crystallization.

Example 6:   Compression molding of porous P4HB

Classified sodium chloride crystals (80-180 μm) were mixed with

30   molten P4HB as described in Examples 4 and 5. The polymer salt ratio can be adjusted to produce the desired porosity, while particle size may be adjusted to produce pores of varying size. The polymer salt mixture was pressed into a thin film using the conditions described in Example 6. After

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allowing the material to solidify, the film was removed from the mylar

backing. The film was exhaustively extracted with water to remove the salt,

leaving a porous film of P4HB. Salt removal was monitored by analysis of

chloride in the supernatant and confirmed by elemental analysis of the

5   leached film (less than 0.5% chloride). Mechanical data for 50% and 80%

porous P4HB (pP4HB50 and pP4HB80, respectively) are shown in Table 1.

Example 7:   Cell seeding of P4HB scaffolds.

Porous P4HB (as described in Example 6) was sterilized by cold

ethylene oxide treatment. It was seeded with ovine vascular cells and

10   cultured *in vitro*. Preliminary data indicated very good attachment of these

cells to the material. This is a further demonstration of the biocompatibility

of this material. The number of cells attached to the material can be

quantified using an assay for DNA and compared with the standard for tissue

engineering scaffolds, PGA mesh.

15   **Example 8:   Fiber orientation.**

Compression molded strips of P4HB were uniaxially stretched. The

sample narrowed and became clear, showing signs of necking. After this

stretching process, the polymer appeared stronger and somewhat more

flexible, demonstrating uniaxial orientation of the sample.

20   **Example 9:   Thermal phase separation method for production of**
                  P4HB foam.

P4HB was dissolved in dioxane at 1 to 5% wt./vol. This polymer

solution was cast as a thick film and solidified by cooling on ice below the

melting point of dioxane. The solvent was evaporated from this solid

25   material at low pressure to yield a porous foam with the approximate

dimensions of the starting thick film. ESEM analysis of this material

showed a highly porous, sponge-like structure. The polymer concentration

and cooling process can be varied to alter the porosity of the foam. Prior to

freezing, the polymer solution can be shaped into a variety of forms, broken

30   up into particulate material or used as a coating. Therefore, this thermal

phase separation technique can be used to produce a great variety of highly

porous, 3-dimensional shapes of P4HB.

Example 10: P4HB coating of a PGA non-woven mesh.

P4HB was dissolved in tetrahydrofuran at 1% wt/vol. A 1 mm thick
non-woven mesh of PGA (Albany International, bulk density 52 mg/cc) was
dipped into this solution so that the air voids were eliminated. The coated
5   mesh was allowed to air dry and the coating procedure was repeated. Light
microscopic and ESEM analyses of the coated mesh showed that during the
drying process the polymer migrated to the fiber intersections, and
functioned to bind the fibers together. This fiber bonding technique was
found to dramatically improve the strength and handleability of the PGA
10   mesh. Tensile testing according to ASTM D638, showed that the tensile
strength, Young's modulus and ultimate elongation of this material were 130
psi, 240 psi and 171%. This was a dramatic improvement over the uncoated
material which was too fragile to test these parameters.

Example 11: P4HB foam coating of a PGA non-woven mesh.

15   P4HB was dissolved in dioxane at 2.5% wt/vol. A 1 mm thick non-
woven mesh of PGA (Albany International, bulk density 52 mg/cc) was
dipped into this solution so that the air voids were eliminated. The coated
mesh was cooled on ice so that the coating solution solidified. The mesh
was freeze-dried to remove the dioxane. Light microscopic analysis of the
20   coated mesh showed that during the freeze-drying process the polymer
formed a web-like foam throughout the PGA mesh. This foamed material
has good handleability. The high surface area and improved mechanical
properties are attractive for a variety of applications.

Example 12: Formation of P4HB Microspheres.

25   P4HB was dissolved in dichloromethane at 1% wt/vol. A 1 ml
volume of this solution was mixed with 5 ml of a 0.5% wt/vol. solution of
sodium dodecylsufate (SDS). The two phase mixture was mechanically
mixed to yield an emulsion. A stream of nitrogen was bubbled through the
mixture for 1 hour with rapid stirring to facilitate removal of the
30   dichloromethane. The mixture was stirred open to the air overnight to allow
for the complete removal of dichloromethane. The resultant suspension
contained P4HB microspheres of about 1-10 μm, as determined under a
phase contrast optical microscope.

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Summary

Polyhydroxyalkanoates such as the homopolymer P4HB and copolymers containing 4HB have physical properties and degradation characteristics which make them very attractive as implants for use in medical applications. These polymers can be fabricated into fibers, sheets, foams, coating, structures, filaments and the like for use of these as implantable medical materials.

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Table 1.   **Thermal and Mechanical Properties of Selected Medical Polymers.**

Polymer	Tm (°C)	Tg (°C)	Tensile Str. (psi)	Modulus (psi)	Elongation (%)	Degradation
[1]P4HB	60	-51	7,500	9,400	1000	depends on config.
[1]pP4HB50[a]	60	-51	895	2155	164	depends on config.
[1]pP4HB80[b]	60	-51	180	257	100	depends on config.
[6]P4HB-3HB 10%	50	-42	9,000	14,500	1080	Not reported
[1]PHB	175	0	4,000	110,000	4	>52 wks
[2]PGA	230	35	10,000	1,000,000	17	8 wks
[3]PDLLA	Am	53	5,000	300,000	5	<8 wks
[3]PLLA	175	55	10,000	500,000	8	>8 wks
[2]DLPLG 50/50	Am	48	7,000	300,000	5	3-8 wks
[5]LDPE			2,000		400-700	Nondegradable
[5]HDPE			4,000		100-1000	Nondegradable
[5]UHMWPE			7,250		450	Nondegradable
PP			4,000	20,000	200-700	Nondegradable
PET			8,500		50	Nondegradable
PTFE			3,000 @ Yield	50,000	300	Nondegradable

[a] pP4HB50, 50% porous P4HB, see example 7.
[b] pP4HB80, 80% porous P4HB, see example 7.

Ref: 1. From this work measured according to ASTMD638 at ambient temperature and a strain rate of 0.05 or 0.1 in./min.. 2 Hutmacher et al. Int. J. Oral Max. Imp. 1996, 11, 667-678. 3 Nobes et al. submitted. 4. Mark, Physical Properties of Polymers Handbook, American Inst. of Physics, Woodbury, New York, 1996. 5. Schwartz, S.S. and Goodman, S. H. Plastic Materials and Processes, Van Nostrand Reinhold Company, New York, 1982. 6. Saito, Y. and Doi, Y. Int. J. Biol. Macromol. (1994) 16: 99-104.

Table 2.   GC analysis of poly(4HB-co-2HB) from MBX1177/pFS30 and MBX184/pFS30.

Strain	4HB, g/L	2HB, g/L	Total PHA, % of dcw[a]	P4HB, % of PHA[b]	P2HB, % of PHA[b]
184/30	2	8	18.3	70.8	19.2 (14.2)[c]
184/30	5	5	47.1	98.8	1.2 (0.9)[c]
1177/30	2	8	13.0	62.3	27.7
1177/30	5	5	40.1	98.9	1.1

[a] dcw: dry cell weight.
[b] Determined by GC analysis. About 20 mg of lyophilized cell mass was subjected to butanolysis at 110°C for 3 hours in 2 mL of a mixture containing (by volume) 90% 1-butanol and 10% concentrated hydrochloric acid, with 2 mg/mL benzoic acid added as an internal standard. The water-soluble components of the resulting mixture were removed by extraction with 3 mL water. The organic phase (1 µL at a split ratio of 1:50 at an overall flow rate of 2 mL/min) was analyzed on an SPB-1 fused silica capillary GC column (30 m; 0.32 mm ID; 0.25 µm film; Supelco; Bellefonte, Pa.) with the following temperature profile: 80 °C, 2 min.; 10 C° per min. to 250 °C; 250 °C, 2 min. The standard used to test for the presence of 4-hydroxybutyrate units in the polymer was γ-butyrolactone. The standard used to test for 2-hydroxybutyrate units in the polymer was sodium (2-hydroxybutyrate).
[c] Percentages in parentheses were determined by GC analysis as above, but after extraction of the polymer into chloroform and subsequent precipitation in ethanol.

Table 3.   GC analysis of poly(4HB-co-2HB) from MBX184/pFS30.

Sample	4HB, g/L	2HB, g/L	Total PHA, % of dcw[a]	P4HB, % of PHA[b]	P2HB, % of PHA[b]
1	2	2	8.2	100	0
2	2	4	5.6	100	0
3	2	6	5.7	84.1	15.9
4	2	8	4.1	54.3	45.7

[a] dcw: dry cell weight.
[b] Determined by GC analysis. See Table 2 for details.

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Table 4. GC analysis of poly(4HB-co-3HB) from MBX1177/pFS30.

Sample	4HB, g/L	3HB, g/L	Total PHA, % of dcw[a]	P4HB, % of PHA[b]	P3HB, % of PHA[b]
3a	4	0.25	49.3	98.0	2.0
_3b	4	0.5	46.7	94:2	5.8
3c	4	0.75	56.6	91.7	8.3
3d	4	1	51.8	89.4	10.6

[a] dcw: dry cell weight.

[b] Determined by GC analysis. See Table 2 for details. The standard used to test for the presence of 4-hydroxybutyrate units in the polymer was γ-butyrolactone. The standard used to test for 3-hydroxybutyrate units in the polymer was poly(3-hydroxybutyrate).

Table 5. Properties of P4HB and P4HB-co-3HB from MBX1177/pFS30.

Sample	%[a] 4HB	%[a] 3HB	Tm[b] (°C)	dH Tm1[b] (J/g)	Tg[b] (°C)	Tx[b] (°C)	Tm2[b] (°C)	Mw[c]
P4HB	100	0	60	45	-51	-16	X	1,000,000
3b	94.2	5.8	47	36	-52	-4	44	1,500,000
3c	91.7	8.3	40	20	-53	nd	39	1,900,000
3d	89.4	10.6	39	17	-53	nd	nd	1,100,000

nd - not detected.

[a] Determined by GC analysis, see Table 2 for details.

[b] Determined by DSC analysis. A Perkin Elmer Pyris 1 differential scanning calorimeter was used. Samples masses were approximately 4-8 mg. The thermal program used was as follows: 25°C, 2 min.; heat to 195°C at 10 C° per min.; hold at 195°C 2 min.; cool to -80°C at 300 C° per min.; hold at -80°C for 2 min.; heat to 195°C at 10 C° per min. The melting temperature (Tm) and the enthalpy of fusion of this melting peak (dHTm1) were determined in the first heating cycle. Glass transition temperature (Tg), crystallization temperature (Tx) and melting temperature (Tm2) were determined during the second heating cycle.

[c] Determined by GPC analysis. Isolated polymers were dissolved in chloroform at approximately 1 mg/mL and samples (50 μL) were chromatographed on a Waters Stryagel HT6E column at a flow rate of 1 mL chloroform per minute at room temperature using a refractive index detector. Molecular masses were determined relative to polystyrene standards of narrow polydispersity.

Table 6:   Degradation of P4HB *in vitro*. Percent original mass
             remaining and molecular mass of film, 50% porous and
             80% porous samples of P4HB incubated in phosphate
             buffer (8 mM sodium phosphate, 2 mM potassium
             phosphate, 140 mM NaCl, 10 mM KCl, pH 7.4, containing
             NaN$_3$ as preservative) at 37°C.

Implan-tation (weeks)	Film Weight % Remaining[a]	Film Molecular Mass[b]	50% Por. Weight % Remaining[a]	50% Por. Molecular Mass[b]	80% Por. Weight % Remaining[a]	80% Por. Molecular Mass[b]
0	108	1144592	96	963145	123	1291117
1	97	1160707	93	1103860	99	968245
2	101	1008496	98	1055614	106	1072328
4	100	887005	96	725089	116	987665
6	109	896521	97	764260	95	1049079
10	92	772485	90	605608	100	727543

[a] Determined by GPC analysis. See Table 3 for details.
[b] Determined by quantitative GC analysis. See Table 2 for details.

Table 7:   Degradation of P4HB *in vivo*. Percent original mass remaining and molecular mass of film, 50% porous and 80% porous samples of P4HB implanted subcutaneously in rats.

Implantation (weeks)	Film Weight % Remaining[a]	Film Molecular Mass[b]	50% Por. Weight % Remaining[a]	50% Por. Molecular Mass[b]	80% Por. Weight % Remaining[a]	80% Por. Molecular Mass[b]
0	108	1144592	96	963145	123	1291117
1	103	1091107	109	1026821	88	1132492
2	95	1054873	94	973830	35	943960
4	92	1007736	73	989629	39	881919
6	90	797170	74	901330	28	689157
10	80	716296	48	647175	0	N/D

N/D Not determined.

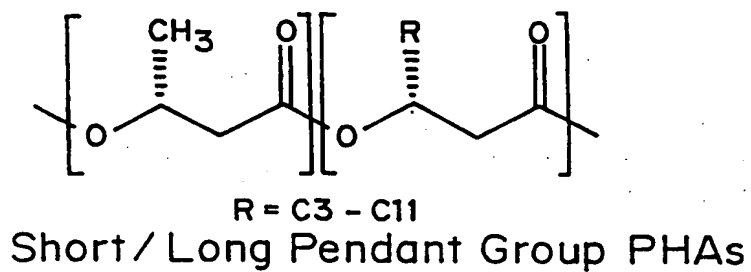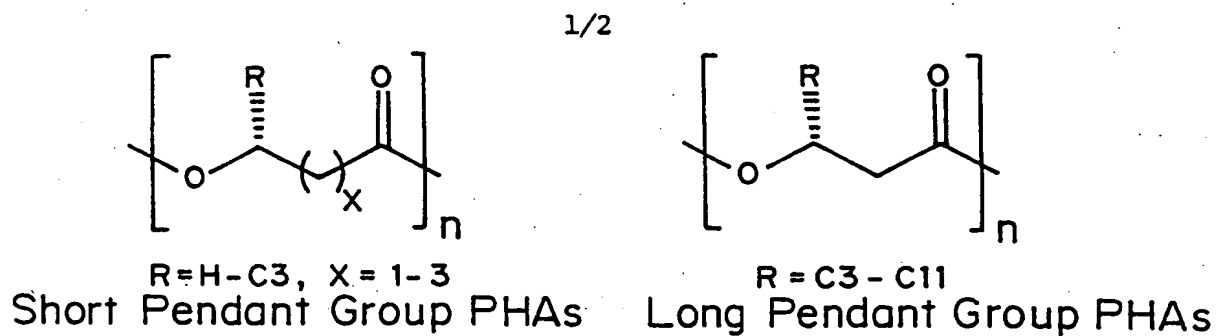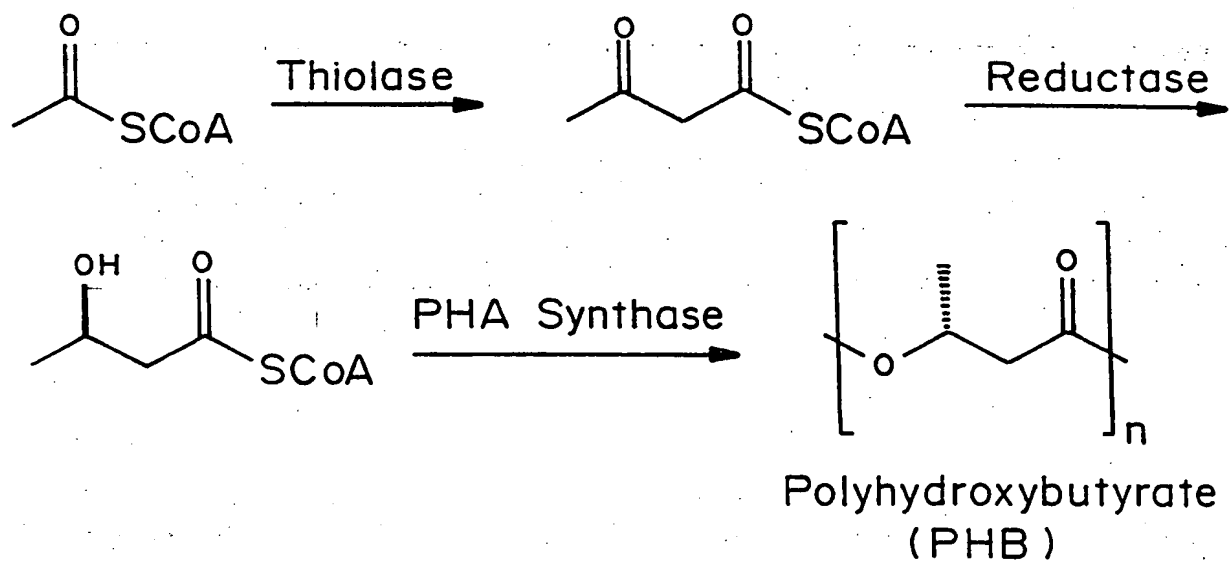[a] Determined by GPC analysis. See Table 3 for details.

[b] Determined by GC analysis. See Table 2 for details. Explants often weighed more than the original implant due to the presence of adherent tissue or coagulated blood. Therefore, the mass of P4HB in the explant was determined by quantitative GC analysis. Weight percent remaining P4HB was taken as this mass divided by original implant.

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We claim:

1.   A biocompatible polyhydroxyalkanoate that has a controlled degradation rate of less than one year under physiological conditions.

2.   The polyhydroxyalkanoate of claim 1 comprising one or more units which alter the chemical stability of the polymer backbone.

3.   The composition of claim 2 wherein the presence of the additional unit(s) promotes chain scission.

4.   The composition of claim 2 wherein the units contain more than two functional groups.

5.   The composition of claim 1 wherein a heteroatom is incorporated into the polymer backbone chain.

6.   The composition of claim 5 wherein the heteroatom is selected from the group consisting of oxygen, sulfur or nitrogen.

7.   The composition of claim 2 wherein the units are incorporated into the polymer backbone with chemical linkages selected from the group consisting of ester, amide, ether, carbamate, anhydride, and carbonate.

8.   The composition of claim 2 wherein the units are selected from the group consisting of 2-hydroxyacids; 2-hydroxyalkoxyacetic acids; amino acids; amino alcohols; diacids; triols; and tetraols.

9.   The composition of claim 8 wherein the 2-hydroxyacids are 2-hydroxyalkanoic acids.

10.   The composition of claim 9 wherein the 2-hydroxyalkanoic acid is lactic acid.

11.   The composition of claim 9 wherein the 2-hydroxyalkanoic acid is glycolic acid.

12.   The composition of claim 8 wherein the 2-hydroxyacids are 2-hydroxyalkenoic acids.

13.   The composition of claim 8 wherein the 2-hydroxyalkoxyacetic acids are selected from the group consisting of 2-hydroxyethoxy acetic acid and 3-hydroxypropoxy acetic acid.

14.   The composition of claim 1 comprising pendant groups that catalyze the degradation of the polymer backbone.

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15.   The composition of claim 14 wherein the pendant groups are selected from acidic and basic groups.

16.   The composition of claim 14 comprising reactant pendant groups that cause polymer chain scission.

17.   The composition of claim 16 wherein the reactant pendant groups are selected from nucleophiles and electrophiles.

18.   The composition of claim 14 wherein the pendant groups are selected from the group consisting of alcohols, acids and amine groups.

19.   The composition of claim 1 comprising additives altering the chemical stability of the polyhydroxyalkanoate.

20.   The composition of claim 19 wherein the additives promote chain scission.

21.   The composition of claim 19 wherein the additives are selected from the group consisting of acids, bases, electrophiles, nucleophiles, plasticizers, polymers, pore forming agents, and agents designed to reduce the polymer crystallinity.

22.   The composition of claim 1 comprising pore forming agents.

23.   The composition of claim 22 wherein the pore forming agents are lyophilizable particles.

24.   The composition of claim 22 wherein the pore forming agents absorb water.

25.   A biocompatible polymeric device for implantation or injection into a patient in need thereof comprising the polymer of any one of claims 1-24.

26.   Methods for preparing the compositions of any one of claims 1-24.

27.   The methods of claim 26 selected from the group consisting of fermentation, enzymatic synthesis, chemical synthesis, melt processing, reactive blending, and recombinant techniques.

28.   The method of claim 27 wherein the composition is derived by fermentation using a co-feed.

29.   The method of claim 27 wherein the fermentation process uses transgenic bacteria.

30.   The methods of claim 27 wherein the chemical synthesis involves insertion, transesterification, and/or ester metathesis.

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31.   Methods for processing the compositions of any one of claims 1-24 into a medical device.

32.   The methods of claim 31 wherein the processing involves a process selected from the group consisting of solvent casting, melt processing, fiber processing, fiber spinning, fiber weaving, extrusion, injection molding, compression molding, lamination, an microparticle formation.

33.   A medical device comprising any one of the polymers of claims 1-24.

34.   The device of claim 33 for use in tissue engineering.

35.   The device of claim 33 comprising a therapeutic agent for use in controlled drug delivery.

36.   The device of claim 33 comprising an imaging agent selected from the group of agents detectable by a method consisting of ultrasound, MRI, CAT, x-ray, and fluorescence.

37.   The device of claim 33 for use in orthopedic applications.

38.   The device of claim 33 wherein the device is porous.

39.   The device of claim 33 in the form of microparticles.

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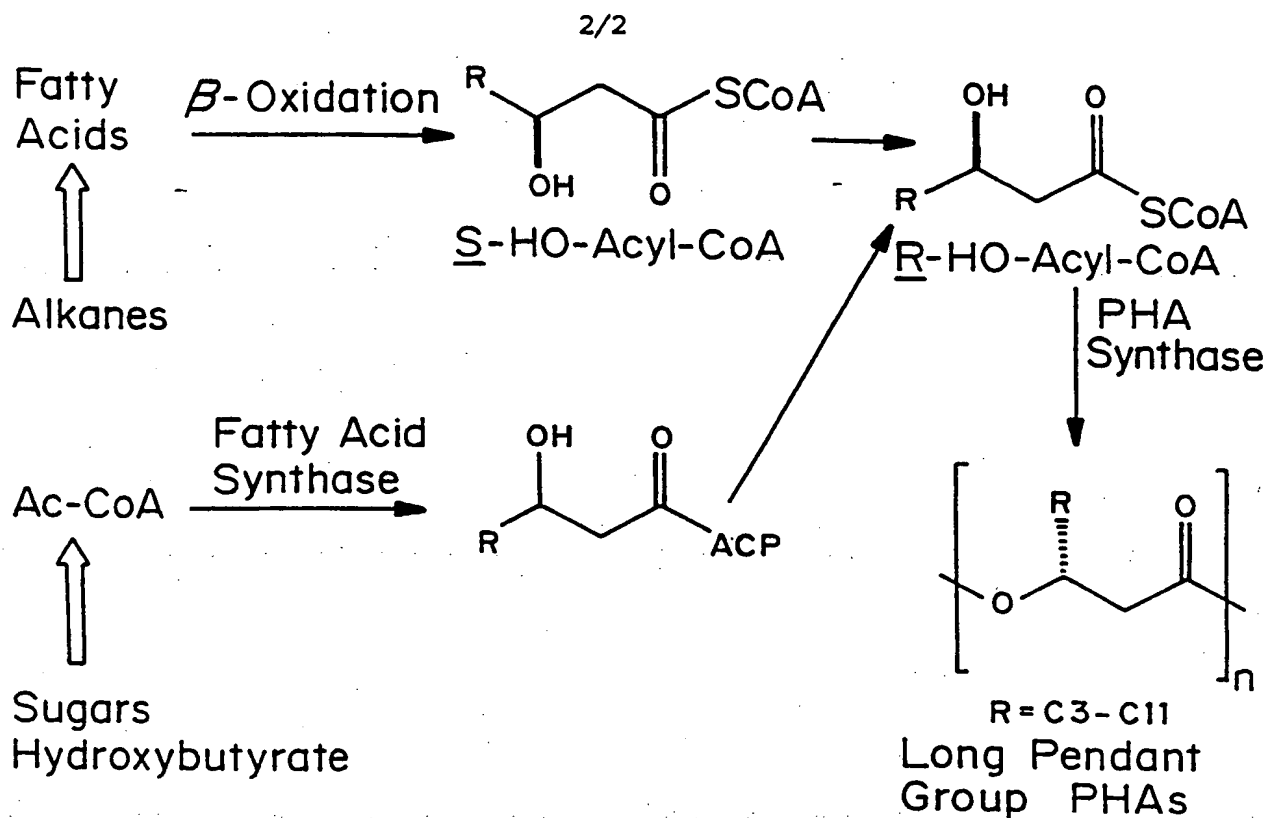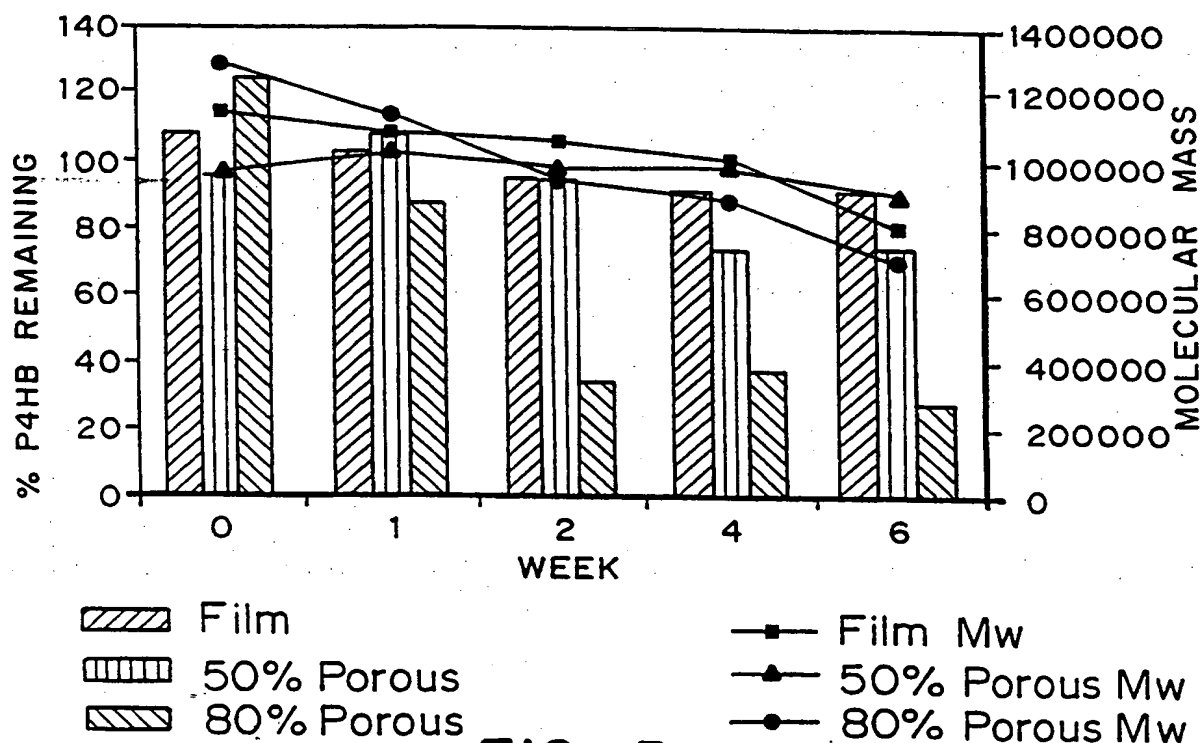R=H−C3, X = 1−3
Short Pendant Group PHAs

R = C3 − C11
Long Pendant Group PHAs

R = C3 − C11
Short / Long Pendant Group PHAs

FIG. 1

Polyhydroxybutyrate (PHB)

FIG. 2A

FIG. 2B

FIG. 3

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C08G63/06   C08G63/91   C08K3/00   C08K5/00   C08J9/26
        C08J9/28   C08L67/04   C12P7/62   A61K47/34   A61L17/00
        A61L25/00   A61L27/00   A61L31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C08G   C08K   C08J   C12P   A61L   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9252 Derwent Publications Ltd., London, GB; Class A23, AN 92-429826 XP002101841 & JP 04 326932 A (NIPPON ZEON KK) , 16 November 1992 see abstract	1-3,5-7, 31-33,38
X	DATABASE WPI Section Ch, Week 9310 Derwent Publications Ltd., London, GB; Class A23, AN 93-079450 XP002101842 & JP 05 023189 A (MITSUBISHI KASEI CORP) , 2 February 1993 see abstract	1-3,5-7, 25-28, 33-35

-/--

X	Further documents are listed in the continuation of box C.		X	Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 May 1999	21/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Krische. D

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DE 39 37 649 A (BOEHRINGER INGELHEIM KG) 16 May 1991	1-3,5-7, 25-27, 33-35,37
Y	see claims 1,5,6,17; examples 2,4,5 see column 4, line 31 - line 49	19-21
Y	A. KISHIDA ET AL.: CHEM. PHARM. BULL., vol. 37, no. 7, 1989, pages 1954-1956, XP002101838 see the whole document	19-21
X	DATABASE WPI Section Ch, Week 9551 Derwent Publications Ltd., London, GB; Class A23, AN 95-399485 XP002101843 & JP 07 275344 A (NIPPON ZEON KK) , 24 October 1995	1-3,5-7, 25,31-34
Y	see abstract	19-22,24
Y	WO 93 20134 A (ALZA CORP) 14 October 1993 see abstract; claim 1 see page 1, line 16 - line 22 see page 10, line 19 - page 11, line 30	19-22,24
X	LEE C W ET AL: "COPOLYMERIZATION OF GAMMA-BUTYROLACTONE AND BETA-BUTYROLACTONE" MACROMOLECULAR CHEMISTRY AND PHYSICS, vol. 198, no. 4, 1 April 1997, pages 1109-1120, XP000677489 see page 1109 see page 1113 - page 1114	1-3,5-7, 26,30
X	Y. SAITO ET AL.: INT. J. BIOL. MACROMOL., vol. 16, no. 2, 1994, pages 99-104, XP002101839 cited in the application see the whole document	1-3,5-7, 26-28
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